WEST

Generate Collection APC and transfection

L12: Entry 2 of 22 File: USPT Apr 4, 2000

DOCUMENT-IDENTIFIER: US 6045802 A
TITLE: Enhanced immune response to an antigen by a composition of a recombinant virus expressing the antigen with a recombinant virus expressing an immunostimulatory molecule

BSPR:
Recombinant vaccines can take many forms. Recombinant proteins can be synthesized by vectors such as baculovirus (an insect vector) or in eukaryotic cells. Synthetic peptides can also serve as immunogens. Peptide vaccines which consist of 9 to several dozen amino acids can take two forms. They can be mixed with adjuvant or they can be used to pulse peripheral blood cells as antigen presenting cells (APCs) for reinfusion into the patient. Recombinant vaccines can also be constructed by inserting the gene which codes for a given tumor associated antigen into a vector. Some of the common vectors used are vaccinia virus, avian pox viruses such as fowlpox or canary pox, BCG, adenovirus and Salmonella. These vectors, each with their advantages and disadvantages are usually employed because of the immunogenicity of their constitutive proteins, thus rendering the protein or epitope of the inserted gene more immunogenic. Recombinant vaccines can also take the form of an anti-idiotype antibody which is directed against a monoclonal antibody prepared against a given tumor associated antigen. Most recently, polynucleotide vaccines have been prepared which consist of naked DNA of a tumor associated gene in a plasmid containing a promoter gene. Whereas all of the above have been analyzed in animal models, very few studies have compared relative efficiencies of one approach versus the other. Clinical trials have now begun using some of these approaches in breast cancer and other carcinoma patients and others will most likely begin in the near future.

BSPR:
There are several antigens that have now been identified for potential use in recombinant vaccines for cancer therapies. The first of these is the c-erbB/2 oncogene which is found to be over expressed in approximately 20-30% of breast tumors (Pietras R J et al. Oncogene (9:1829-1838, 1994). It has been shown (Bernards R et al. Proc. Natl. Acad. Sci. USA 84:6854-6858, 1987) that the point mutated c-erbB/2 oncogene in rats, when inserted into vaccinia virus, is immunogenic and can lead to anti-tumor effects. The human c-erbB/2, however, is not mutated. It has recently been shown (Disis M L et al., Cancer Res. 54:1071-1076, 1994)), that this gene contains several epitopes which appear to generate human T-cell responses in vitro. The point mutated p53 oncogene, also found in many human breast tumors has been shown

to be a potential target for cytotoxic T-cells (Yanuck M et al. Cancer Res. 53:3257-3261, 1993). Clinical studies are now beginning in which peptides reflecting specific point mutations are being pulsed with human peripheral blood lymphocytes (PBLs) and readministered to patients. The breast cancer mucin, MUC-1 or DF3, represents a differentiation antigen of the breast (Abe M and Kufe D, Proc. Natl. Acad. Sci. USA 90:282-286, 1993). While MUC-1 is expressed in a range of normal epithelial tissues, it appears to be uniquely glycosylated in breast cancer tissue. The tandem repeat of the core protein of the MUC-1 mucin has been reported to be immunogenic in humans (Barnd D L et al., Proc. Natl. Acad. Sci. USA 86:7159-7163, 1989) in that lymph nodes of breast cancer patients contain T-cells which can be activated by MUC-1 peptides in an non-MHC restricted manner. It has also been shown (Rughetti A et al., Cancer Res. 53:2457-2459, 1993) that ovarian cancer patients can make antibody responses to this region. Animal models in which the MUC-1 gene has been inserted into vaccinia virus have been reported (Hareuveni M et al., Proc. Natl. Acad. Sci. USA 87:9498-9502, 1990; Hareuveni et al., Vaccine 9:618-626, 1991). A clinical trial in which MUC-1 peptide is being pulsed with human PBLs is currently underway in breast cancer patients. Another mucin that represents a potential target for cancer therapy is TAG-72 which is found on approximately 70-80% of human breast cancers (Thor A et al., Cancer Res. 46:3118-3124, 1986).

BSPR:
The human carcinoembryonic antigen (CEA) also represents a potential target molecule for the immunotherapy of a range of human carcinomas including colorectal, gastric, pancreatic, breast, and non-small cell carcinomas (Robbins P F et al., Int. J. Cancer 53:892-897, 1993; Esteban J M et al., Cancer 74:1575-1583, 1994). Experimental studies have shown that anti-idiotype antibodies directed against anti-CEA monoclonal antibodies can elicit immune responses in mice (Bhattacharya-Chatterjee M et al., Int. Rev. Immuno. 7:289-302, 1991). Clinical studies using this anti-idiotype antibody are currently in progress. A recombinant vaccine has also been developed in which the CEA gene has been inserted into vaccinia virus (Kantor J. et al., J. Natl. cancer Inst. 84:1084-1091, 1992). A Phase I clinical trial involving this vaccine has just been completed.

BSPR:
Vaccinia viruses have been extensively used in humans and the use of a vaccina based vaccine against smallpox has led to the worldwide eradication of this disease (reviewed in reference Moss, B. Science 252:1662-1667, 1991). Vaccinia viruses have the advantages of low cost, heat stability and a simple method of administration. Attempts have been made to develop vaccinia virus vectors for the prevention of other diseases.

BSPR:
Vaccina virus is a member of the pox virus family of cytoplasmic DNA viruses. DNA recombination occurs during replication of pox viruses and this has been used to insert DNA into the viral genome. Recombinant vaccina virus expression vectors have been

extensively described. These vectors can confer cellular immunity against a variety of foreign gene products and can protect against infectious diseases in several animal models. Recombinant vaccina viruses have been used in human clinical trials as well. Cooney et al immunized 35 healthy HIV seronegative males with a recombinant vaccinia virus expressing the gp160 envelope gene of HIV (Cooney, E.. The Lancet 337:567-572, 1991). Graham et al randomized 36 volunteers to receive either recombinant vaccinia virus containing the gp160 HIV envelope protein or control vaccinia virus (Graham, B. S. et al J. Infect. Dis. 166:244-252, 1992). Phase I studies using recombinant vaccinia virus have begun in patients with metastatic melanoma using a recombinant virus expressing the p97 melanoma antigen (Estin, C. D. et al Proc. Nat'l Acad. Sci. 85:1052-1056, 1988) and a Phase I trial to use recombinant vaccinia virus expressing the human carcinoembryonic antigen in patients with advanced breast, lung or colorectal carcinoma has just been completed. In these trials, vaccinia virus is administered by intradermal scarification and side effects have been minimal including local skin irritation, lymphadenopathy and transient flu-like symptoms.

BSPR:
Another considerable advantage of avian pox virus is that there may be little or no cross-reactivity with vaccinia virus and thus previously vaccinated humans may not have preexisting immune reactivity to fowlpox virus proteins.

DRPR:
FIG. 4 shows each treatment group of 5 C57BL/6 mice immunized with recombinant vaccinia viruses encoding the genes for either human carcinoembryonic antigen gene (rV-CEA), murine B7-2 (rV-B7), or the wild type strain of vaccinia virus (V-Wyeth). Each mouse was administered 1.times.10.sup.7 plaque forming units by tail scarification in the following ratios: 1:1 rV-CEA/rV-B7 (5.times.10.sup.6 PFU rV-CEA+5.times.10.sup.6 PFU rV-B7); 1:1 V-Wyeth/rV-B7 (5.times.10.sup.6 PFU V-Wyeth+5.times.10.sup.6 PFU rV-B7); or 1:1 rV-CEA/V-Wyeth (5.times.10.sup.6 PFU rV-CEA+5.times.10.sup.6 PFU V-Wyeth). Three spleens were removed and pooled from each treatment group 14 days after immunization and a standard 5 day lymphoproliferative assay was performed as previously described (Kantor, et al. JNCI, 84:1084, 1992). Purified T cells were tested for their proliferative capacity against a Baculovirus produced recombinant CEA at 100 .mu.g/ml. Stimulation index was calculated in relationship to the cells reactivity to media (background).

DRPR:
FIG. 5 shows each treatment group of 5 C57BL/6 mice immunized with recombinant vaccinia viruses encoding the genes for either human carcinoembryonic antigen gene (rV-CEA), murine B7-2 (rV-B7), or the wild type strain of vaccinia virus (V-Wyeth). Each mouse was administered 1.times.10.sup.7 plaque forming units by tail scarification in the following ratios: 1:3 rV-CEA/rV-B7 (2.5.times.10.sup.6 PFU rV-CEA+7.5.times.10.sup.6 PFU rV-B7); 1:3 V-Wyeth/rV-B7 (2.5.times.10.sup.6 PFU V-Wyeth+7.5.times.10.sup.6 PFU rV-B7); or 1:3 rV-CEA/V-Wyeth (2.5.times.10.sup.6 PFU rV-CEA+7.5.times.10.sup.6 PFU V-Wyeth). Three spleens were

removed and pooled 14 days after immunization and a standard 5 day lymphoproliferative assay was performed as previously described (Kantor, et al. JNCI, 84:1084, 1992). Purified T-cells were tested for their proliferative capacity against a Baculovirus produced recombinant CEA at 100 .mu.g/ml. Stimulation index was calculated in relationship to the cells reactivity to media (background).

DEPR:
In one embodiment, the composition comprises a recombinant virus comprising a vaccinia virus genome or portions thereof, the nucleic acid sequence encoding CEA and a recombinant virus comprising the nucleic acid sequence encoding the immunostimulatory molecule, B 7.1 alone or in combination with the nucleic acid sequence encoding the immunostimulatory molecule, B7.2, or a recombinant virus containing both the genes for a tumor antigen and a immunostimulatory molecule.

DEPR:
The present invention also encompasses a recombinant virus comprising the virus genome or portion thereof, and one or more nucleic acid sequences encoding one or more B7 molecules, preferably a recombinant vaccinia virus expressing B7-1 and/or B7-2. The rapid infection of tumor cells with these recombinant viruses demonstrates that vaccinia can authentically express these proteins and that they are functional molecules. Weakly immunogenic syngeneic tumors expressing these recombinant molecules are rejected by immunocompetent hosts.

DEPR:
In a specific embodiment recombinant virus is a recombinant vaccinia virus containing B7.1 and a recombinant vaccinia virus containing B7.2 (designated rV-B7-1 and rV-B7-2, respectively).

DEPR:
Virus that may be used in the present invention are those in which a portion of the genome can be deleted to introduce new genes without destroying infectivity of the virus. The virus vector of the present invention is a nonpathogenic virus. In one embodiment the virus vector has a tropism for a specific cell type in the mammal. In another embodiment, the virus vector of the present invention is able to infect professional antigen presenting cells such as dendritic cells and macrophages. In yet another embodiment of the present invention, the virus vector is able to infect any cell in the mammal. The virus vector may also infect tumor cells.

DEPR:
The virus of the present invention include but is not limited to Poxvirus such as vaccinia virus, fowlpox virus and a highly attenuated vaccinia virus (MVA), adenovirus, baculovirus and the like.

DEPR:
The vaccinia virus genome is known in the art. It is composed of a HIND F13L region, TK region, and an HA region. The recombinant vaccinia virus has been used in the art to incorporate an

exogenous gene for expression of the exogenous gene product (Perkus et al. Science 229:981-984, 1985; Kaufman et al. Int. J. Cancer 48:900-907, 1991; Moss Science 252:1662, 1991)

DEPR:
A gene encoding an antigen of a disease state or disease causing agent may be incorporated into the HIND F13L region or alternatively incorporated into the TK region of recombinant vaccinia virus vector or other nonessential regions of the vaccinia virus genome. Likewise, a gene encoding an immunostimulatory molecule may be incorporated into the HIND F13L region or the TK region of recombinant vaccinia virus vector.

DEPR:
Baxby and Paoletti (Vaccine 10:8-9, 1992) disclose the construction and use as a vector, of the non-replicating poxvirus, including canarypox virus, fowlpox virus and other avian species which may be used as a viral vector in the present invention.

DEPR:
Co-stimulatory molecules of the B7 family (namely B7.1, B7.2, and possibly B7.3) represent a more recently discovered, but important group of molecules. B7.1 and B7.2 are both member of the Ig gene superfamily. These molecules are present on macrophages, dendritic cells, monocytes, i.e., antigen presenting cells (APCs). If a lymphocyte encounters an antigen alone, with co-stimulation by B7.1, it will respond with either anergy, or apoptosis (programmed cell death); if the co-stimulatory signal is provided it will respond with clonal expansion against the target antigen. No significant amplification of the immune response against a given antigen occurs without co-stimulation (June et al. (Immunology Today 15:321-331, 1994); Chen et al. (Immunology Today 14:483-486); Townsend et al. (Science 259:368-370)). Freeman et al. (J. Immunol. 143:2714-2722, 1989) report cloning and sequencing of B7.1 gene. Azuma et al. (Nature 366:76-79, 1993) report cloning and sequencing B7.2 gene.

DEPR:
In one embodiment the B7.1 gene or the B7.2 gene was inserted into vaccinia virus. In another embodiment, the CEA gene and the IL-2 gene were both inserted into a single vaccinia virus. The rV-CEA/.sub.n IL-2 (ATCC Designation VR 2480), rV-CEA-T108 (ATCC Designation No. VR 2481), rV-.sub.m B7-2 (ATCC Designation VR 2482); and rV-.sub.m B7-1 (ATCC Designation VR 2483) were deposited with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Md. on Oct. 3, 1994 under the terms of the Budapest Treaty.

DEPR:
In one embodiment, the CEA antibodies of this invention generated by immunization with a composition comprising recombinant vaccinia virus expressing CEA and a recombinant vaccinia virus expressing B7.1 are used to assess the presence of the CEA antigen from a tissue biopsy of a mammal afflicted with a cancer expressing CEA using immunocytochemistry. Such assessment of the delineation of the CEA antigen in a diseased tissue can be used

to prognose the progression of the disease in a mammal afflicted with the disease or the efficacy of immunotherapy. Conventional methods for immunohistochemistry are described in (Harlow and Lane (eds) (1988) In "Antibodies A Laboratory Manual", Cold Spinning Harbor Press, Cold Spring Harbor, New York; Ausbel et al. (eds) (1987). In Current Protocols In Molecular Biology, John Wiley and Sons (New York, N.Y.).

DEPR:
A 1,125 bp DNA fragment encoding the entire open reading frame of murine B7-1 and a 942 bp DNA fragment encoding the entire open reading frame of murine B7-2 was amplified by reverse transcriptase PCR (Geneamp RNA PCR Kit, Perkin Elmer, Norwalk, Conn.) from total RNA extracted from the murine B-cell line, A20 (TIB 208, ATCC, Rockville, Md.). The sequences of the B7 inserts were shown to be identical to the published sequences (Freeman, G. J. et al. J. Exp. Med. 174:625-631, 1991; Freeman, G. J. et al. Science 262:813-960, 1993). The DNA fragments were ligated separately into the Kpn-1/Xho-1 restriction enzymes sites of the vaccinia virus transfer vector PT116, provided by Therion Biologics (Cambridge, Mass.) which contains the Escherichia Coli Lac Z gene for the selection of the recombinant viruses. Recombinant viruses were derived as previously described (Kaufman, H. et al. Int. J. of Cancer 48:900-907, 1991). Recombinant clones were selected by growth on BSC-1 cells (CC126, ATCC) in the presence of 5-bromo-4-chloro-3-indolyl-beta D galactosidase (X-Gal). Appropriate blue recombinant clones were purified by 5 rounds of plaque purification and grown into a higher titer lysate. Virus for inoculation was grown in spinner cultures of HeLa cells, directly pelleted by centrifugation, and purified over 20%-40% sucrose gradients (Moss, B. Current Protocols in Molecular Biology 2.16.15.1-16. 18. 9, 1993).

DEPR:
Confluent BSC-1 cells were infected with either wild-type vaccinia virus or recombinant vaccinia viruses containing the murine B7-1 or B7-2 genes (designated V-Wyeth, rV-B7-1 or rV-B7-2) at an MOI of 10 for 4 hours. Protein was extracted and analyzed as described previously (cantor, J. et al. J. Nat'l Cancer Inst. 84:1084-1091, 1992). Recombinant B7-1 or B7-2 protein was detected by incubating western blots with anti-B7-1 (purified Rat-Anti mouse B7/BB1) or anti-B7-2 (Rat-anti-mouse B7-2 (GL-1) monoclonal antibodies (Pharmingen, San Diego, Calif.), followed by incubation with Goat-anti-rat conjugated to horseradish peroxidase (HRP, Kirkegaard & Perry Laboratories, Gaithersburg, Md.) and developed as per manufacturers instructions.

DEPR:
The cDNA fragments encoding the open reading frames of murine B7-1 and B7-2 were obtained by reverse transcriptase PCR using B7-1 specific oligonucleotide primers 5' GGTACCATGGCTTGCAATTGTCAGTTG 3' (SEQ ID NO.:1), 5' CTCGAGCTAAAGGAAGACGGTCTG 3' (SEQ ID No.:2), and B7-2 specific primers 5' GGTACCGAAGCACCCACGATGGAC 3' (SEQ ID No.: 3), 5' CTCGAGTCACTCTGCATTTGGTTTTGC 3' (SEQ ID No.:4) and ligated into the vaccinia virus transfer vector PT116. This vector contains a

strong vaccinia virus immediate early promoter (designated P40) upstream of the multiple cloning site to drive the synthesis of the inserted gene product. The ligation and orientation of the B7 DNA fragments, as well as promoter position were verified by PCR and sequencing. The chimeric vector construct was inserted into the vaccinia virus genomic Hind III M site by homologous recombination as previously reported (Kaufman H. et al. Int. J. Cancer 48:900-907, 1991), and confirmed by Southern analyses with .sup.32 P radiolabeled B7-1 or B7-2 DNA as a probe (data not shown). The entire cDNA sequences of B7-1 and B7-2 in the vaccinia virus clones were shown to be identical to the published sequences (Freeman, G. J. et al. J. Exp. Med. 174:625-631, 1991; Freeman, G. J. et al. Science 262:813-960; 1993).

DEPR:
Cell surface expression of B7-1 or B7-2 recombinant proteins was examined by flow cytometry. FIG. 1A illustrates that uninfected BSC-1 cells (FIG. 1A) do not react with either B7(BB1) or B7-2 (G1-1) antibodies (98.5% of the cells are negative with a mean fluorescence of 5.22). Similarly, cells infected with wild type vaccinia (V-Wyeth, FIG. 1B), failed to react with either of these two antibodies (97.7% of the cells are negative with a mean fluorescence of 5.43). BSC-1 cells infected with rV-B7-1 (FIG. 1C) react strongly with the B7/BB1 antibody (97.5% of the cells are positive with a mean fluorescence of 2513.68). Cells infected with rV-B7-2 (FIG. 1D) react strongly with the B7-2 (G1-1) antibody (98.8% of the cells are positive with a mean fluorescence of 1802.30). There was no reactivity between antibody B7/BB1 and cells infected with rV-B7-2, and no reactivity of antibody GL-1 with cells infected with rv-B7-1. These studies thus demonstrate that a recombinant vaccinia virus can express the B7-1 and B7-2 molecules on the cell surface at 3-6 hours post infection. Lysis of infected cells usually does not occur for 24-48 hours (Moss, B. Current Protocols in Molecular Biology 2.16.15.1-16.18.9, 1993).

DEPR:
In systems using retroviral vectors for introduction of B7 genes, it has been shown that concurrent administration to mice of B7 expressing tumor cells on one flank and administration of B7 negative tumor cells on the opposite flank would prevent the growth of both tumor populations (Chen, L. et al. Cell 71:1093-1102, 1992). In other studies, antitumor activity to B7 negative cells was demonstrated by the multiple intraperitoneal injections of B7 expressing tumor cells ten days following the subcutaneous administration of B7 negative primary tumor (Li, Y. et al. J. Immunol 153:421-428, 1994). The study described here was designed to determine if long lasting immunity to tumor cells could be induced by immunization with rV-B7 infected tumor cells. Mice administered MC38 cells infected with rV-B7-1 or rV-B7-2 remained tumor free for in at least 40 days (FIGS. 2C and 2D) and were then challenged on the opposite flank with 3.times.10.sup.5 uninfected (B7 negative) MC38 cells (FIGS. 3B and 3C). The injection of these MC38 cells into naive mice (FIG. 3A) resulted in palpable tumor formation in all animals within 7 days, with progressive tumor growth throughout the duration of this experiment. Average tumor volume in this control group at 21 days

post tumor transplant was 2436.+-.858 mm.sup.3. Mice that had 40 days previously been administered tumor infected with rV-B7-1 were also challenged with uninfected MC38 cells (FIG. 3B). The formation of these tumors was delayed, and the growth rate was substantially reduced, with an average tumor volume of 372.+-.106 mm.sup.3 at day 21. Similarly, mice that had been administered rV-B7-2 infected tumors, when challenged with MC38 cells, displayed a substantial reduction in growth of tumor cells. Average tumor volume in this group was 197.+-.161 mm.sup.3 at day 21. Tumor growth was thus reduced by >90% in animals previously receiving tumors expressing B7-1 or B7-2 via recombinant vaccinia virus infection. This was of interest in light of the fact that only one immunization was administered 40 days prior to tumor challenge, and that the mice were challenged with a relatively large tumor burden. This implies that a memory immune response against a rejection antigen on MC38 tumor cells is being induced by the injection of rV-B7 infected tumor cells. The above method of treatment may be modified to include multiple inoculations with B7 expressing tumor cells, and enhancement of T-ell activation with immunostimulatory molecules including cytokines such as IL-2.

DEPR:
Previous studies have demonstrated that the introduction of B7 into tumor cells via transduction with retroviral vectors such as PLNSX, PLNCX or PLXSN can confer immunogenicity to those tumors (Chen, L. et al. J. Exp. Med. 179:523-532, 1994; Dohring, C. et al. Int. J. Cancer 57:754-759, 1994). These methods of B7 introduction have a potential limitation for clinical applications due to a relatively low efficiency of infection of retroviral vectors and the consequent prolonged amount of time required to drug select and expand the B7 positive tumor cells. As an alternative, the studies reported here have demonstrated the development of recombinant vaccinia viruses expressing the genes for the costimulatory molecules B7-1 and B7-2. These recombinant vaccinia constructs infect tumor cells rapidly (1-4 hours) and express recombinant protein with high efficiency (over 97% of cells, FIGS. 1C and 1D, respectively). Infected cells were shown to authentically synthesize the recombinant proteins, leading to antitumor effects. These studies thus present data in an experimental system for the insertion of B7 genes into vaccinia virus vectors with implications for potential immunotherapeutic applications.

DEPR:
The present invention comprises a composition of rV-B7 in combination with a recombinant vaccinia virus expressing a human tumor associated antigen. The composition of the present invention when coinoculated into a host to enhance the systemic T-cell immune response to that human tumor associated antigen.

DEPR:
In the first study, rV-CEA and rV-B7 were mixed at one to one ratios, i.e., 5.times.10.sup.6 pfu of B7 and 5.times.10.sup.6 pfu of rV-CEA were mixed and coadministered to groups of three mice by tail scarification. Spleens were removed 14 days post-immunization as a source of lymphocytes. As controls, three

other groups of mice were used: (a) non-vaccinated mice, (b) mice receiving 5.times.10.sup.6 pfu of rV-CEA and 5.times.10.sup.6 pfu of wild type vaccinia (designated V-Wyeth), and (c) mice receiving 5.times.10.sup.6 pfu of V-Wyeth and 5.times.10.sup.6 pfu of rV-B7. Thus, all vaccinated mice received a total of 10.sup.7 pfu of vaccinia virus and in all three groups, ratios of vaccinia viruses were 1 to 1. As can be seen in FIG. 4, after one administration of rV-CEA plus V-Wyeth, mice did mount an immune response specific for CEA, albeit low. The immune assay employed was a lymphoproliferative assay which as been described previously (Kantor et al., J. Natl. Cancer Inst., 84:1084-1092, 1992) and the target antigen used was recombinant CEA derived from baculovirus. As can be seen in FIG. 4, the addition of rV-B7 to rV-CEA enhanced the specific immune response several fold. In contrast, the addition of rV-B7 to the control V-Wyeth had no effect on enhancing the CEA specific immune response.

DEPR:
These results indicate that simply mixing a rV-containing a human associated gene with rV-B7 can lead to coinfection and coexpression on antigen presenting cells so as to enhance specific T-cell responses for the human tumor associated antigen. Moreover, it appears that the ratios of rV-B7 and the rV-containing human associated gene used may be an important factor in optimizing T-cell activation to a human tumor associated gene product or indeed any other gene product one wished to induce or enhance immunity to.

DEPR:
C57BL/6 mice were immunized with 1.times.10.sup.7 PFU total virus with various ratios of either: V-Wyeth; rV-CEA:V-Wyeth; Wyeth:rV-B7; or rV-CEA:rV-B7, and CEA specific lymphoproliferation was analyzed as previously described (Kantor, J. et al J. Nat'l Cancer Inst. 84:1084-1091, 1992). Briefly, spleens were removed 14 days following immunization and mechanically dispersed through 70 .mu.m cell strainers (Falcon, Becton Dickinson, Franklin Lakes, N.J.) to isolate single cell suspensions. Erythrocytes and dead cells were removed by centrifugation over a Ficoll-Hypaque gradient (density=1.119 g/ml) (Sigma Chemical Co., St. Louis, Mo.). Populations consisting of approximately 95% T-cells were obtained by passage of splenic mononuclear cells over nylon wool columns (Robbins Scientific Corp., Sunnyvale, Calif.). To evaluate CEA specific lymphoproliferation, T-cells were added at 10.sup.5 /well in 96 well flat bottomed plates (Costar, Cambridge, Mass.). Antigen presenting cells consisted of irradiated (2000 rads) naive syngeneic splenocytes added at 5.times.10.sup.5 /well. Stimulated wells received purified human CEA (100-12.5 .mu.g/ml) (Vitro Diagnostics, Denver, Colo.); ovalbumin as a negative control (100 .mu.g/ml); UV-inactivated V-Wyeth (2.times.10.sup.7 PFU/ml) as a recall antigen or Con-A (2 .mu.g/ml) as a T-cell positive control. Control wells received T-cells, APC's and media only. Cells in all wells were cultured in a total volume of 200 .mu.l of complete media (CM), [RPMI 1640 with fetal calf serum (10%); glutamine (2 mM), sodium pyruvate (1 mM), Hepes (7 mM), gentamicin (50 .mu.g/ml), 2-mercaptoethanol (50 .mu.M), and non-essential amino acids (0.1 mM), (Biofluids, Rockville, Md.)]

for 5 days. Cells were labeled for the final 12-18 h of the incubation with 1 .mu.Ci/well [.sup.3 H]thymidine (New England Nuclear, Wilmington, Del.) and harvested with a PHD cell harvester (Cambridge Technology, Cambridge, Mass.). The incorporated radioactivity was measured by liquid scintillation counting (LS 6000IC; Beckman, Duarte, Calif.). The results from triplicate wells were averaged and are reported as stimulation index (SI) as calculated: SI=[CPM (stimulated wells)]/[CPM (control wells)].

DEPR:
A 786 bp DNA fragment encoding the entire open reading frame of human prostate specific antigen was amplified by reverse transcriptase PCR (GeneAmp RNA PCR Kit, Perkin Elmer, Norwalk, Conn.) from total RNA extracted from the human metastatic prostate adenocarcinoma cell line, LNCaPFGC (CRL 1740, American Type Culture Collection (ATCC), Rockville, Md.). The predicted amino acid sequence derived from the PSA coding sequence was shown to be nearly identical to the published sequence (Lundwall et al, FEBS Letters 214:317-322, 1987) differing only in a change from asparagine to tyrosine at position 220. The PSA DNA fragment, containing the entire coding sequence for PSA, 41 nucleotides of the 5' untranslated region, and 520 nucleotides of the 3' untranslated region, was ligated into the Xba I restriction enzyme site of the vaccinia virus transfer vector pT116. The resulting plasmid, designated pT1001, contained the PSA gene under the control of the vaccinia virus 40K promoter (Gritz et al, J. Virology 64:5948-5957, 1990) and the Escherichia coli Lac Z gene under the control of the fowlpox virus C1 promoter (Jenkins et al, AIDS Research and Human Retroviruses 7:991-998, 1991). The foreign genes were flanked by DNA sequences from the Hind III M region of the vaccinia genome. A plaque-purified isolate from the Wyeth (New York City Board of Health) strain of vaccinia was used as the parental virus in the construction of recombinant vaccinia virus. The generation of recombinant virus was accomplished via homologous recombination between vaccinia sequences in the Wyeth vaccinia genome and the corresponding sequences in pT1001 in vaccinia infected RK.sub.13 cells (CCL 37, ATCC) transfected with pT1001. Recombinant clones were identified and selected by growth on RK.sub.13 cells (CCL 37, ATCC) in the presence of 5-bromo-4-chloro-3-indoly-beta-D-galactopyranoside (X-Gal) as described previously (Panicali et al, Gene 47:193-199, 1986; Kaufman et al, Int. J. Cancer 48:900-907, 1991). Appropriate blue recombinant clones were purified by four rounds of plaque purification. Virus stocks were prepared by clarifying infected RK.sub.13 cell lysates followed by centrifugation through a 36% sucrose cushion.

DEPR:
Confluent BSC-40 cells were infected with either parental wild type vaccinia virus (designated V-Wyeth) or recombinant vaccinia-PSA (designated rV-PSA) at an MOI of 1 in Dulbecco's Modified Eagle's Medium containing 2% fetal bovine serum. After an overnight infection, the medium was removed from the cells, and an aliquot was methanol precipitated to assay for the presence of secreted PSA. The infected cells were lysed in

hypotonic lysis buffer (150 mM NaCl, 0.05% EDTA, 10 mM KCl, 1 mM PMSF) and then sonicated. Cell lysates and culture media were electrophoresed on an SDS-10% acrylamide gel. The proteins were transblotted to nitrocellulose, and the blot was incubated with a rabbit antibody specific for PSA (PO798, Sigma Chemical Co., St. Louis, Mo.) for 4 hours at ambient temperature, washed, and then incubated with goat anti-rabbit phosphatase-labeled secondary antibody (A P, Kirkegaard & Perry Laboratories, Gaithersburg, Md.) and developed according to the manufacture's instructions.

DEPR:
The cDNA fragment encoding the open reading frame of human PSA was obtained by reverse transcriptase PCR using PSA specific oligonucleotide primers 5' TCTAGAAGCCCCAAGCTTACCACCTGCA 3' (SEQ ID NO:5), 5'TCTAGATCAGGGGGTTGGCCACGATGGTGTCCTTGATCCACT 3' (SEQ ID NO:6), and ligated into vaccinia virus transfer vector pT116. This vector contains a strong vaccinia virus early/late promoter (designated 40K) upstream of the multiple cloning site to drive the synthesis of the inserted gene product. The ligation and orientation of the PSA DNA fragment, as well as promoter position were verified by PCR and sequencing. The chimeric vector construct was inserted into the vaccinia virus genome Hind III M site by homologous recombination as previously reported (Kaufman et al, Int. J. Cancer, 48:900-907, 1991) and confirmed by Southern analysis probing with .sup.32 P radiolabeled DNA corresponding to PSA sequences and vaccinia sequences in the Hind III M region (data not shown).

DEPR:
Expression of recombinant PSA protein was confirmed by western blot analysis of supernatant fluids and protein extracts from rV-PSA infected BSC 40 cells. These cells are routinely used for the evaluation of recombinant vaccinia products (Earl et al, Current Protocols in Molecular Biol., 2.16.15.1-16.18.9, 1993). Incubation of cell supernatant blots from rV-PSA infected cells with rabbit anti-PSA antibody revealed a single immunoreactive polypeptide of approximately 33,000 daltons (data not shown). Similarly, incubation of protein extract blots from rV-PSA infected cells revealed a single band of the same molecular weight (data not shown). This is consistent with the predicted size of the PSA molecule (Armbruster et al, Clin. Chem. 39:181-195, 1993; Wang et al Methods in Cancer Research, 19:179-197, 1982). Cell supernatant blots or protein extract blots from cells infected with parental strain V-Wyeth remained negative for expression of PSA. These results thus demonstrate that a recombinant vaccinia virus can faithfully express the human PSA gene product.

DEPL:
Recombinant Vaccinia Virus

DEPL:
Recombinant Vaccinia Virus

DEPC:
Induction of Enhanced a T-cell Immune Response to a Human Tumor Associated Antigen by Mixing a Recombinant Vaccinia Virus

Expressing the Tumor Associated Antigen With a Recombinant Vaccinia Virus Expressing the B7 Co-Stimulatory Molecule

CLPR:
8. The composition according to claim 1, 5, 6 or 7 wherein the first recombinant virus, the second recombinant virus, or the first and second recombinant viruses is selected from the group consisting of retrovirus, fowlpox, canarypox, swinepox, adenovirus, vaccinia virus and poliovirus.

CLPR:
12. The composition according to claim 1, 5, 6 or 7 wherein the first recombinant virus, the second recombinant virus, or the first and second recombinant virus is vaccinia virus.

ORPL:
J. Ruby et al., 1990, "Response of monkeys to vaccination with recombinant vaccinia virus which coexpress HIV gp160 and human interleukin-2", Immunol. Cell Biol., 68, 113-117.

ORPL:
Kantor et al, Immunogenicity and Safety of a Recombinant Vaccinia Virus Vaccine Expressing the Carcinoembryonic Antigen Gene in a Nonhuman Primate, Cancer Research, vol. 52, No. 24, 1992, pp. 6917-6925.

ORPL:
Expression of Proteins in Mammalian Cells Using Vaccinia Viral Vectors, Overview of the Vaccinia Virus Expression System, Current Protocols in Molecular Biology, Section IV, Unit 16.15 to 16.19.9, 1991.

ORPL:
Kantor et al, Antitumor Activity and Immune Responses Induced by a Recombinant Carcinoembryonic Antigen-Vaccinia Virus Vaccine, Journal of the National Cancer Institute, Jul. 15, 1992, vol. 84, No. 14, pp. 1084-1091.

ORPL:
Kaufman et al, A Recombinant Vaccinia Virus Expressing Human Carcinoembryonic Antigen (CEA), Int. J. Cancer, 48, pp. 900-907, 1991.

ORPL:
Cochran et al, In Vitro Mutagenesis of the Promoter Region for a Vaccinia Virus Gene: Evidence for Tandem Early and Late Regulatory Signals, Journal of Virology, vol. 54, (No. 1) Apr. 1985, pp. 30-37.

ORPL:
Flexner et al, Expression of Human Interleukin-2 by Live Recombinant Vaccinia Virus, Vaccines, 1987, PP, 380-383.

ORPL:
Smith et al, Vaccinia Virus Expression Vectors: Construction Properties and Applications, Biotechniques, Nov./Dec. 1994, pp. 306-312.

ORPL:
Hodge et al, The Admixture of a Recombinant Vaccinia Virus Containing the Gene for the Costimulatory Molecule B7 and a Recombinant Vaccinia Virus Containing a Tumor Associated Antigen Gene Results in Enhanced Specific T-Cell Responses and Antitumor Immunity, Cancer Research, vol. 55 (16) pp. 3598-3603, Aug. 15, 1995.